

AMERICAN DENDROBATID GROUP

Newsletter No. 18

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The purpose of the ADG is to develop better communication between Dendrobatid breeders in North America. It is designed, by its format and bi-monthly distribution, to provide current information and new developments in the hobby. We hope that this will aid us in solving some of the problems which confront us all. This newsletter appears bimonthly at a cost \$10.00 per calander year. Back issues for 1992 are available for \$5.00; back issues for 1993 are available for \$10.00.

Subscriptions, comments, etc. should be sent to Charles Powell (2932 Sunburst Dr., San Jose, CA 95111 Tel.: (408) 363-0926) .

NOTES FROM THE EDITOR

First I'd like to thank everyone who has contributed articles, pictures, comments, etc. this year: Will Brown, Steve Grenard, Brian Monk, Ed Oshaben, Malcolm Peaker, Alicia Pinzari, Rex Lee Searcey, Jack Wattlely, and Kevin Wright. You have helped make this Newsletter what it is - Great! My heart felt thanks to all.

This issue of the Newsletter completes the 1994 year and dues are due for 1995. You will not receive any more issues until membership is paid. Membership will remain at \$10 through 1995. But, we are requesting donations to help pay for the purchase of a label printer. Dues only covers costs related to printing and mailing and not the time needed to put the Newsletter together and get it in the mail, not to mention answer letter and phone calls. Since our membership is growing so fast this is taking up more and more time. So, for those who can afford it we are requesting \$3 or more in order to purchase a label printer. If everyone on the mailing list would send in at least \$3 we would have just about enough to purchase the printer which will help save time in producing the Newsletter.

Enclosed with this months Newsletter is the membership list. We had originally planned to include a list of frogs kept by each member but only a few members let me know which species they keep and becuase of worry about possible government intervention we will not be listing that information. Look to the Adds section of the Newsletter for this information. If you have an extra frog of one sex or another - please list it in the breeding loan/request section. Please keep the membership list confidential.

KITCHEN COUNTER FROG PATHOLOGY: PART 2

Brian Monk

Continued from last issue — Now your ready to start the post-mortem. Before you begin cutting, examine the outside of the frog thoroughly. Note everything! Does the frog appear emaciated and thin? Is it fat? Is its muscle tone and mass similar to other healthy frogs? Try to bend the long bones of the limbs with your fingers. Are they firm and hard,

or abnormally soft and flexible, indicating some type of bone disease? Does the skin have any obvious pigment changes? Ulcers? Cuts, bruises, abrasions, lumps, etc.? If lesions are present, then take a closer look. Obtain a microscopic sample by taking a swab or the edge of your blade and scraping or rubbing across the particular lesion. If there are no lesions, then obtain a general swab or scrape of a part of the frog's skin, preferably in an area that has not been pressed against the storage container. Remove the material to a slide, place a few drops of saline on it, and examine the unstained slide under the microscope. This is called a wet mount. (Warning: do not use the oil immersion lens for this type of slide! You won't see anything, and you might ruin your lens!) Make two additional slides, and then let them air dry. Fix and stain one with Wright's stain, and Gram's stain the other. The Wright's stain will allow you to see big cells, parasites, protozoans, and some bacteria. The Gram's stain will allow you to determine the type of bacteria present. If you can only do one stain, do the Wright's stain.

Next, open the specimen's mouth and examine the inside with a light. Check for obvious lesions. Sometimes a frog will regurgitate its stomach contents into its esophagus and mouth. Use a swab or the cleaned edge of your blade and make two wet mounts from the mouth as above. After you have observed them microscopically, stain them and examine them as above. As a warning, all animals have normal flora and fauna, which are microorganisms that coexist with the creature. These microorganisms generally do no harm, and do not cause disease. Just because you find a microbe does not mean you have found your killer — examine the entire frog, make notes, and then think about what might be the cause.

Now it is time to open the frog. Take your blade and carefully make a lengthwise incision from the middle of the frog's lower jaw all the way to the anus, which should be located where the back legs meet. Make two crosswise incisions: one along the belly near the back legs and one across the upper chest near the front legs (Fig. 1).

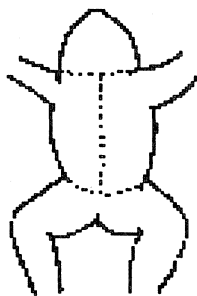


Fig. 1 Dotted lines indicate incisions

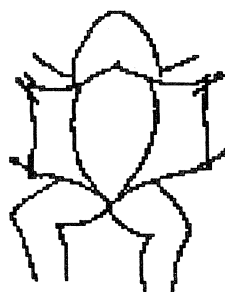


Fig. 2 "Opened" frog showing inner cavity

Be careful not to damage any underlying tissues. Carefully fold back the skin and pin it out of the way, exposing the thoracic and abdominal cavity (Fig. 2). Obtain a drop or two of the

fluid present in the abdominal cavity, and make slides as above. Note if there seems to be an excessive amount of fluid in the abdomen. The intestines, stomach, and liver should be immediately visible (Fig. 3). Follow the path of the intestine up to the stomach and down to the cloaca. Note anything that seems unusual.

The liver should be a relatively large organ, a little larger than the stomach, with several lobes, located in the cranial (towards the head) area of the abdominal cavity. It

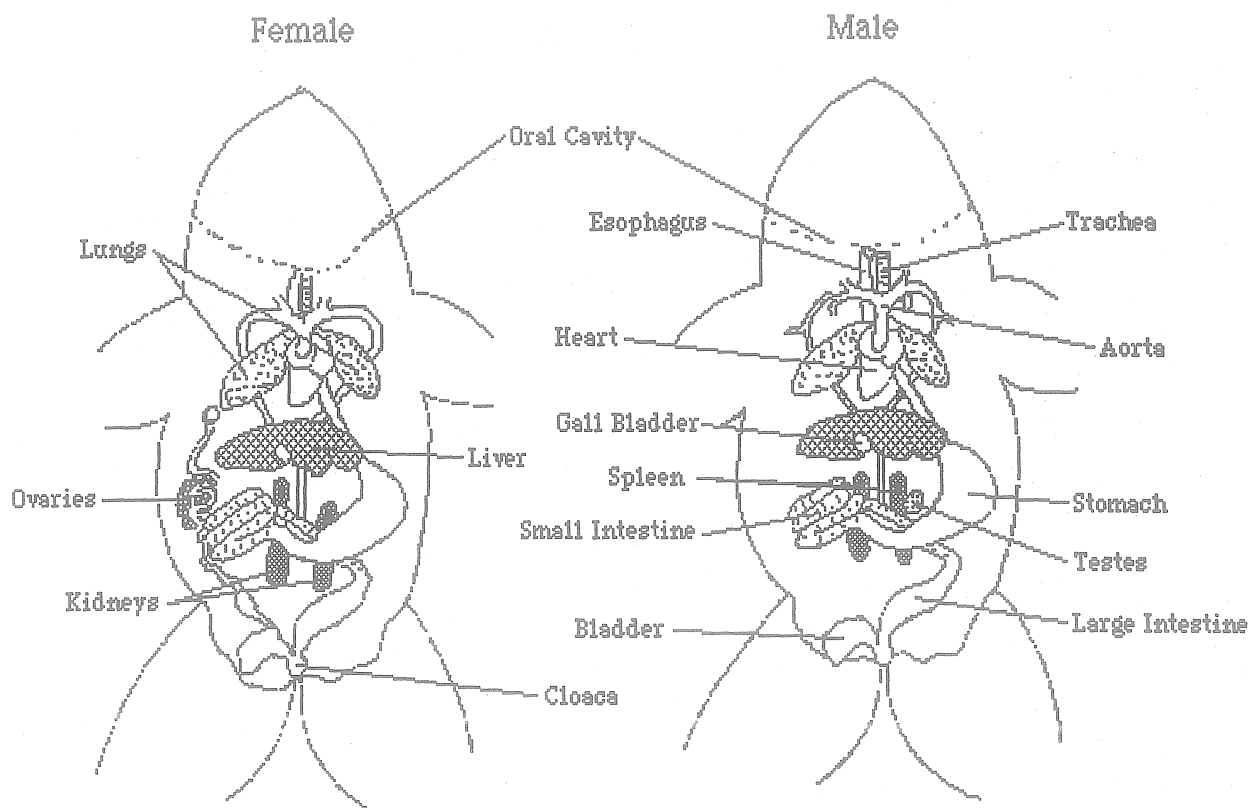


Fig. 3 Representative internal structures of a male and female anuran

should be firm, smooth, and have a uniform dark reddish-brown coloration. Note any abnormal size, texture, or coloration. Cut out a small section of the liver and examine its inner morphology, which should be uniform in texture and color. Place the piece of liver in a specimen container filled with 10% formalin. This will fix the tissue and prepare it for histological examination, which I will explain at the end of this article. All other tissues, as well as the entire frog, should be sampled in this manner and placed into 10% formalin. In most cases a diseased liver will be larger than average size and/or mottled with different colors, but it will exhibit some lack of uniformity either in texture or coloration. A diseased liver can indicate any number of pathological conditions, which primarily include improper diet, poisoning, viral infections, and age; however, a diseased liver can be secondary

to and indicative of pathological conditions elsewhere.

The intestines should be pale white/yellow, and should be free of any obvious physical lesions. Their contents are sometimes visible from the outside, though not always. The contents of the intestines should move up and down the tract with gentle pressure. Note any hard, knotty areas that may indicate a blockage. Look for small areas of hemorrhage, which appear as small reddish-brown spots, or other areas of discoloration. Do not confuse lesions or areas of hemorrhage with the normal blood vessels that supply the tract. If possible, remove the lower intestinal tract by cutting at the middle of the small intestine and just before the cloaca and lifting it out of the body. Do not let the contents fall into the open abdomen. Incise the tube for part of its length and swab the inside, making separate slides of the contents of the small and large intestines. The inner wall of the intestine should be similar to the outer wall in color and have many folds in the small intestine, or be relatively smooth in the large intestine. Examine the slides as wet mounts, remembering that this area of the body will have the greatest populations of normal flora, and that you should see some microbes. However, abnormal conditions include parasites (worm-like), parasite eggs (round to ovoid, surrounded by a wall), and some protozoans (single-celled organisms that may be amoeboid, or have many moving hairs or filaments). Stain the slides after they are dry (preferably both Wright's and Gram stains). Generally, Gram positive bacteria are normal, whereas Gram negative bacteria are indicative of some type of disease condition.

Behind the intestines, you should find the kidneys, reproductive organs, and fat bodies. The kidneys should be 6-8 times as long as they are broad, and one should be located on either side of the body, with two major blood vessels running between them. The kidneys should be smooth, and of a uniform reddish-brown color. Cut a section out of the kidneys, and examine it for any obvious lesions. Lumps, rough texture, or differences in coloration can indicate kidney disease, which can result directly from poisoning or dehydration, and can be secondary to other pathological conditions such as infection or liver disease. The fat bodies should be finger-like projections of yellowish color, located around the reproductive structures. They should be greatest in size in a well-fed, non-breeding frog. Small fat bodies can indicate breeding activity or malnutrition. However, an absence of fat bodies may also be normal. The reproductive organs of the male should consist of small, round to ovoid testes positioned over the kidneys, or in the female a relatively long oviduct running in many folds beside the kidneys, possibly with a mass of unfertilized eggs in several different states of maturity within the ovary.

The next major step in the necropsy is to examine the organs of the thoracic cavity (Fig. 3). This should be done by cutting through the pectoral girdle (chest) as far to the sides as possible, making two vertical lines from the opening of the abdominal cavity to the shoulders. Then cut two horizontal lines, connecting the two vertical incisions. Be careful when cutting not to damage the underlying organs. Remove the tissue plate, and examine the internal contents of the thoracic cavity. Swab the inside of the cavity and make slides to be examined and stained. The heart should be within a grayish, translucent sack (pericardium) made of tough connective tissue. Insert a needle into one of the three chambers of the heart (two atria, one ventricle) and draw out some blood. This may not be

possible in a long-dead frog. Examine the blood on a slide, then make a Wright's stain and examine again. The red blood cells should be greatest in abundance. They are elliptical discs with three parts: a thick "rim" or edge, a thick center (caused by the nucleus), and a thin section between the center and edge. Most white blood cells are larger than red blood cells, are less abundant, and appear more bluish after Wright's staining. Some may have a grainy appearance, and some may be relatively clear of granules. A large number of white blood cells can indicate infection, but normal values of red and white cell numbers are unfortunately unknown.

The lungs are paired organs behind the heart. They are sac-like, conical structures, the inner surface being divided by blood vessels, connective tissue, and smooth muscle. They should be relatively giving to the touch — extreme rigidity could indicate disease. Obvious lesions include excessive fluid present within the lung spaces, hemorrhaging, cysts, and parasites. Section the lungs, and obtain a swab of the cut surface. Make two wet mount slides, examine them, and then stain them as above. Bacteria, protozoans, and cellular debris (particularly white blood cells) can be indicative of disease.

The last part of the post-mortem is the diagnosis. Making a diagnosis can be confusing, but if you have collected a broad range of data from the examination, noting both normal and abnormal findings, then it should be fairly easy to narrow down the possible causes of death. Diagnoses can range from little more than an educated guess to a definite identification of a specific pathogen or disease process. Most diagnoses will fall somewhere between these two extremes.

Bacterial infections are typically systemic (whole body), and are normally caused by an opportunistic bacteria that is present as normal flora on the frog. Initial infections can result from undue stress. Specific bacterial infections of one or two organ systems may also be caused by opportunistic pathogens, though typically they result from exposure to more insidious bacterial species that are normally not found on the frog.

Protozoans are a normal component of the normal fauna of amphibians in general, particularly in the lower digestive tract. However, general overabundance or presence in a different tissue or organ (especially the blood) is indicative of a disease process. Larger parasites can include nematode worms, annelid worms, helminth (flat) worms, or arthropods. Their presence is almost universal in wild populations. However, the stress of captivity can often allow these parasites to increase their numbers to the point where the frog suffers from disease. Adult nematodes, trematodes, and tapeworms will normally inhabit the lumen of the intestinal tract. Other helminths may inhabit the various organs of the frog, with some inhabiting more than one tissue depending on their maturity. Parasite eggs are usually found in the feces. Arthropod parasites can be mites and ticks, associated with the skin or lungs; or the pentastomid worms, which inhabit the intestines or lungs.

The very nature of a poison-dart frog can make doing a necropsy extremely frustrating, if not near impossible. These frogs are small, and their size alone can make a dissecting microscope a necessary piece of equipment. In this article I am intentionally neglecting to mention anything but a crude, "what does it look like" type of microbiology. In fact, the proper culturing of possible microbial pathogens is extremely important in

making an accurate diagnosis of the cause of death — yet the average hobbyist has little experience in pathogenic microbiology. Another important component of an accurate post-mortem is histopathology, which is the examination of the microscopic anatomy of tissues for abnormalities. Unfortunately, there is little histopathological data available for amphibians. Tissue samples can be sent through your veterinarian to professional histopathologists, but their experience with amphibians is limited. Finally, there is not space enough in this article to describe even the most common problems of the major organ systems of a relatively complex vertebrate like a frog. You may find something obviously wrong with your frog's liver, for example, but not be able to deduce why it is diseased. However, this does not diminish the importance of post-mortem diagnosis.

As I stated before, the literature concerning captive amphibian husbandry is depauperate in veterinary information. We can help to rectify this situation! Frogs die, and they are replaced with new frogs. Unfortunately, I can envision a day when a frog dies, and there are no replacements. If this day is to be avoided, then proper guidelines for veterinary care of amphibians must be established and made available. Knowledge of the pathological processes is crucial to these guidelines, making post-mortem examinations a necessity. If we are to consider ourselves responsible collectors and breeders, then we should help to provide this knowledge, regardless of the cost in the short run. If we do not, then the long term costs might include the extinction of these frogs which we prize so dearly.

Currently, I am trying to organize a database concerning the histopathology of dart-frogs, as well as other amphibian species. Unfortunately, I am limited in this project by the availability of specimens. If anyone would like to send me specimens and tissue samples of the dart frogs they have examined post-mortem, I would gladly pay for shipping, as well as provide you with any histopathology data that I discover. At this time, I have the resources of the Virginia-Maryland Regional College of Veterinary Medicine at my disposal, but I greatly need the support of professional and amateur collectors of amphibians. Any questions and all material can be directed to the address or phone number mentioned at the beginning of this article. College of Veterinary Medicine at my disposal, but I greatly need the support of professional and amateur collectors of amphibians.

HELPFUL HINTS

If you've been looking for a source for springtail cultures. Now you can try S. S. Enterprises (P. O. Box 37027, Barton P. O., Hamilton, Ontario, Canada L8L 8E9). Starter cultures are \$7.00 postage paid anywhere in Canada and the continental US.

ADDS:

Starting with the next issue we will be including business card adds. Rates are \$10 per issue or \$50 per year. If you are interested please contact the Newsletter editor. All other adds for sale of frogs, requests or offering of breeding loans are free to members.

For Sale

<i>Dendrobates auratus</i> 'Hawaii'	\$25 ea.	Eric Anderson
<i>Dendrobates leucomelas</i> 'Orange'	\$60 ea.	12231 Newberry Rd.
<i>Dendrobates tinctorius</i> 'Cobalt'	\$40 ea.	Gainesville, FL 32607
<i>Dendrobates tinctorius</i> 'Brazil'	\$60 ea.	
(lots of yellow)		
<i>Epipedobates tricolor</i> (3 morphs)	\$30 to \$50 ea.	
<i>Dendrobates leucomelas</i> 'Orange'	\$70 ea.	Jeffrey Lee
		Department of Chemistry
		Duke University
		Durham, NC 27708
		(919) 382-3059
<i>Dendrobates leucomelas</i>	\$40 to \$60	Anthony Leiro
		402 Holly Lane
		Chapel Hill, NC 27514
		(919) 929-3522
<i>Phyllobates vittatus</i>	\$40 ea.	Larry J. Marshall
(nice color)		1239 Park Ave.
		Chicago Heights, IL 60411
		(708) 754-7692
<i>Dendrobates tricolor</i>	\$40 ea.	Patrick Nabors
(brick red with light blue stripes)		St. Louis Lizard Co.
		9849 Manchester
		St. Louis, MO 63119
		(800) 962-7280
<i>Dendrobates auratus</i> 'Zwartgroene'	\$50 ea.	Charles Powell
(a Panamian form which is 80% black)		2932 Sunburst Dr.
<i>Epipedobates tricolor</i> 'Santa Isabel'		San Jose, CA 95111
(bright red and white form)		(408) 363-0926
tadpoles	\$25 ea.	
juvenile frogs (small)	\$50 ea.	
<i>Epipedobates trivittatus</i>	\$75 ea.	Lex Thomas
		(904) 375-5689

Dendrobates azureus
(F1 from wild caught adults)

\$150 ea.

Jack H. Wattley
2500 Sea Island Dr.
Ft. Lauderdale, FL 33301
(305) 436-5011
FAX (305) 463-4716

Giant flightless fruitflies (*Drosophila hydeni*) and wingless fruitflies (*D. melanogaster*) cultures for sale. Cultures produce 500 to 1,000 flies over 6 week period. Member price \$7.00 includes postage (regular \$10.00). Fruit fly food medium makes 30 vials. Member price \$7.50 postage paid (regular \$10.50). Specific instructions included. Loren Testa (433 College St., Woodland, CA 95695 Tel. (916) 666-0520 Weekdays and Saturday 10 to 3). VISA/MC

Reptile Specialities (John Uhern, 10051 Commerce, Tujunga, CA 91042 Tel. (818) 352-1796; Fax (818) 353-7381) have various captive breed Dendrobatids imported for sale. Write or call for information.

Wanted:

Dendrobates imitator

Larry J. Marshall
1239 Park Ave.
Chicago Heights, IL 60411
(708) 754-7692

Dendrobates leucomelas - male

Brice Noonan
2580 53rd Terrace SW
Naples, FL 33999
813) 455 5385

NEW MEMBERS

Ray Anderson (Texas)
Glen Arrowsmith (California)
Robert Gale Breene, III (American Tarantula Society, Texas)
Will Brown (Indiana)
Andy Grubbs (Texas)
Kevin Hansen (California)
Frank Henderson (New Jersey)
William Lutz (Iowa)
Ian Maes (Nevada)
Eric McCraw (North Carolina)
Harold Michaels (Nevada)
Thomas Morgan (Illinois)
James Onuffer (California)
Eric Pflaging & Penny Parker (Hillside Herps, Florida)

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